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D. Flanagan

PI - Signature Date

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Progress During Year 1 (2/15/92 - 2/14/93)

Summary

During this year the following projects have been worked on:

1. Various paromomycin sulfate topical formulations were evaluated with regard to their physical stability. Some of the stability samples were sent to WRAIR for animal testing to determine whether there was sufficient drug remaining to maintain efficacy.

2. Initial studies were conducted on the solubility and stability of alkyl aminophenones and alkyl hydroxyaminophenones to prepare oral liquid and solid formulations for animal studies on their ability to induce methemoglobin.

3. Initial discussions were conducted on the development of topical skin protectant (TSP) formulations.

4. Specifications for the preparation of the best paromomycin sulfate topical formulation (Formulation 232) were delineated. Further physical stability information was collected for Formulation 232 and Modified 232 at lower temperatures.

5. Initial preparations (20 gram) were made of a number of the topical skin protectant (TSP) formulations and sent to ICD for evaluation.

6. A batch of about 4000 antipyrine capsules (250 mg) was produced.

These projects are described in more detail below.

Paromomycin Topical Formulations

During this year various paromomycin sulfate topical formulations were prepared and evaluated with regard to their physical characteristics. The formulations with the most favorable characteristics were sent to WRAIR for animal testing against topical leishmaniasis. Below is a summary of the formulations prepared:

1. The following are the most promising paromomycin sulfate formulations of 25 that were tested:

Formulation 232

Paromomycin sulfate	15.0%
Gentamicin sulfate	0.5%
Water	16.7%
Aquaphilic Ointment with 10% urea	67.8%

Method: 1. Weigh paromomycin sulfate, gentamicin sulfate and Aquaphilic Ointment.

2. Heat Aquaphilic Ointment to 70°C on a hot plate.

3. Dissolve paromomycin sulfate and gentamicin sulfate in the 70°C water by stirring until all solid dissolves.

4. Pour aqueous solution into the heated ointment, stir with a mechanical stirrer until it reaches room temperature.

Nonionic Emulsion Base (Formulation 2)

Paromomycin sulfate	15.0%
Gentamicin sulfate	0.5%
Cetyl	18.0%
Mineral oil, light	15.4%
Span 80	3.0%
Tween	3.0%
Water	47.6%
Methyl paraben	0.1%
Butyl paraben	0.4%

Method: 1. Dissolve paromomycin sulfate, gentamicin sulfate, Tween 80, methyl paraben and butyl paraben into water by heating to about 70°C on a hot plate and stirring until all solids dissolve.

2. Heat cetyl alcohol, light mineral oil and Span 80 on a hot plate to 70°C.

3. Gently add the warm aqueous phase into the oil phase with stirring, remove from heat and stir until the base cools to room temperature.

Result: A homogeneous cream is obtained.

5.0% HPMC K15M gel with 5.0% paromomycin sulfate

Paromomycin sulfate	5.0%
Gentamicin sulfate	0.5%
Methocel K15M	5.0%
Water	89.30%
Methyl paraben	0.18%
Ethyl paraben	0.02%

Method: 1. Dissolve paromomycin sulfate and gentamicin sulfate in water (use half of the total water for the formulation), heat on a hot plate until all solids dissolve.

2. Disperse Methocel into 70°C water (use half of the water for the formulation).

3. Put the dispersed Methocel in an ice bath.

4. Gradually pour drug solution into the cool dispersed Methocel, stir to mix the two portions.

Result: A thick gel is formed with no precipitation from the preservatives.

Physical Stability of Paromomycin Sulfate Topical Formulations

The topical paromomycin sulfate formulations prepared in April, 1992 for animal testing were stored at six temperatures and monitored for viscosity, phase separation and color changes. The six storage temperatures were 3-5°C (refrigerator), 10-12°C, 23-25°C (room temperature), 30°C, 37°C and 45°C. Below is a summary of the observed changes in these formulations over a period of eight weeks:

Formulation 232 (active)

1 day - no changes at 37°C and 45°C.
- 30°C - no changes.

2 weeks - some color change at 45°C and a small amount of water separated out.
37°C - a small amount of water separated out.
30°C - no changes.

4 weeks - at 37°C and 45°C the formulation is stiff when cooled to room temperature and the color is darker than at 2 weeks.
30°C - no changes.

6 weeks - at 37°C and 45°C the formulation is stiff when cooled to room temperature and the color is darker than at 4 weeks.
30°C - no changes.

8 weeks - at 37°C and 45°C the formulation is stiff when cooled to room temperature and the color is darker than at 6 weeks.
at 23-25°C and 30°C - slight color changes.
at 3-5°C and 10-12°C - some separation of water at the bottom of formulation which can be re-mixed.

Modified 232 (active)

1 day - at 37°C and 45°C the viscosity is high and color turned to yellow.
- 30°C - no changes.

2 weeks - at 37°C and 45°C the formulation is very fluid but no phase separation; more color change than at 1 day.
30°C - no changes.

4 weeks - at 37°C and 45°C are the same as at 2 weeks but more color change.
30°C - no changes.

6 weeks - at 37°C and 45°C are the same as at 4 weeks but more color change.
30°C - no changes.

8 weeks - at 37°C and 45°C are the same as at 6 weeks but more color change.
30°C - no changes.

Nonionic Emulsion Base (active)

1 day - at 37°C and 45°C show phase separation and color turned to brown.
- 30°C - no changes.

2 weeks - at 37°C and 45°C - completely deteriorated..
at 30°C - slight oil separation.
23-25°C - no changes.

4 weeks - at 30°C more phase separation and color is yellow.
23-25°C - no changes.

6 weeks - at 30°C - the same as 4 weeks but more color change.
23-25°C - no changes.

8 weeks - at 30°C - the same as 6 weeks but more color change and the color is uneven with the color darker at the top.
23-25°C - no changes.

Aqueous Gel (active)

1 day - at 37°C and 45°C liquid phase and gelling agent separate into two phases.
- 30°C - no changes.

2 weeks - at 37°C and 45°C - completely deteriorated..
at 30°C - viscosity change and slight color change.
23-25°C - no viscosity changes but slight color change.

4 weeks - at 30°C - decreased viscosity and more color change.
23-25°C - no viscosity changes but more color change.

6 weeks - at 30°C - same as 4 weeks but more color change.
23-25°C - same as 4 weeks but more color change.

8 weeks - at 30°C - same as 6 weeks but more color change.
23-25°C - same as 6 weeks but more color change.

Placebo Formulations

Formulation 232: does not hold up at 37°C and 45°C beyond 4 weeks; at all other temperatures it shows no significant changes.

Modified 232: no significant changes except a small viscosity decrease at the higher temperatures.

Nonionic Emulsion: does not hold up at 37°C and 45°C beyond 2 weeks; at all other temperatures it shows no significant changes.

Aqueous Gel: shows significant phase separation at 2 weeks for 37°C and 45°C but other temperatures show no significant changes.

Conclusions:

All of the ointment-type bases (Formulation 232, Modified 232 and the Nonionic Emulsion Base) appear to maintain their physical properties at ambient temperature and below ambient temperatures. The aqueous gel formulation shows a continuous color change over the time period studied which probably indicating reduced chemical stability of the paromomycin in this formulation. All formulations show significant color change, which probably indicates degradation of paromomycin at temperatures above, ambient (37° C and 45°C). The physical properties of these formulations also deteriorate at elevated temperature more rapidly than at ambient or below. The significant of the elevated temperature results as an indication of room temperature stability is of limited value. Real time room temperature and sub-ambient stability data is of most significance. A stability-indicating assay of paromomycin potency is required to positively establish the stability of the drug in these topical bases.

Further Evaluation of Paromomycin Topical Formulation Stability Samples
Some of the paromomycin stability samples were sent on 8/5/92 to WRAIR for animal testing to determine if there was sufficient drug remaining to show efficacy. The following ointments were supplied:

1. Formulation 232 stability samples

   a. 3-5°C sample, started 4/30/92; removed 8/5/92 (1 jar)
   b. 37°C sample, started 4/30/92; removed 8/5/92 (1 jar)
   c. 45°C sample, started 4/30/92; removed 6/30/92 (1 jar)

2. Modified Formula 232 stability samples

   a. 3-5°C sample, started 4/30/92; removed 8/5/92 (1 jar)
   b. 37°C sample, started 4/30/92; removed 8/5/92 (1 jar)
   c. 45°C sample, started 4/30/92; removed 6/30/92 (1 jar)

   Note - for these stability samples, the 3-5°C and 45°C samples are only 30 grams in size while all of the other samples are 60 grams in size.

3. Formulation 232 samples, prepared on 7/31/92 (2 jars)

4. Modified Formula 232 samples, prepared on 7/31/92 (2 jars)

Solubility of Aminophenones in Various Solvents

The solubility of the heptano-hydroxy-derivative (WR 272677AA) and the octano-hydroxy-derivative (WR 271159AA) were investigated in various solvents suitable for intravenous injection. It was noted that the powdered heptano-derivative is considerably darker in color than the octano-derivative. There may be a difference in purity of these two compounds. In solution, the heptano-derivative gives a yellow color while the octano-derivative solutions are colorless when completely dissolved. With the addition of base (NaOH) it was noted that the solutions of both derivatives turn yellow (deeper yellow for the heptano-derivative). This color change is presumably due to ionization of the hydroxylamine group.

The goal was to prepare solutions that were approximately 20 mg in 10 ml of solvent.

The octano-derivative looks like it almost completely dissolves but the solution is hazy indicating colloidal-sized undissolved material. The heptano-derivative shows the best solubility in propylene glycol but it also gives a hazy solution; in the other two solvents, there is quite visible undissolved particles at 2 mg/ml..

Both propylene glycol and PEG 400 appear in IV formulations at levels up to 50%. These injection formulations are painful and can cause vascular irritation with prolonged infusion. Triacetin is soluble in water to about 7% and has been proposed as an IM injection vehicle.

Presumably it will allow faster release of drugs from the injection site. Ethanol has also been used at levels of 10-20% in IV formulations.

One common characteristic of both compounds is that addition of more solvent does not make the haziness disappear completely. Additional solvent dilutes the haze but does not seem to make more compound dissolve. We suspect that this behavior may be due to impurities in both compounds there are insoluble in these solvents. If this is not the case then both compounds are exhibiting solubility behavior that requires further characterization.

Topical Skin Protectant (TSP) Considerations

After review of the information supplied on the TSP formulations containing Teflon particles dispersed in the perfluorinated polyether oil, we have concluded that we can prepare small as well as larger quantities of this formulation with equipment that we have in our laboratories or in Pharmaceutical Service. We have a Vertishear mixer/homogenizer which will act like the Polytron unit described in preparation procedure. In Pharmaceutical Service there is a large steam-heated mixing tank (Groen) that will hold 100 gallons (~ 400 liters). This is mixed with a Gifford-Wood homogenizer (5 hp). We also have smaller mixing tanks with smaller homogenizers. We also have a Hobart mixer with a 140-quart stainless steel mixing bowl for low shear topical formulation production.

The following questions need to be answered to proceed with further development of these formulations in our facilities:

1. Has the particle size of the Teflon been determined? If so, by what means? Does the mixing/dispersing process further reduce the particle size? Are the Teflon particles known to be of regular (i.e., spherical) or irregular shape?

2. What are the specifications on the oil used? What is the chemical nature of the oil and its viscosity/molecular weight? Does the oil have any chemical stability problems?

3. What is the viscosity of the final product and how is the viscosity determined?

4. Is heat required in the manufacturing process? Is there any dispersing/wetting agent used in the manufacture? Are there any other additives in the formulation?

5. Are there any analytical tests that have been performed on the final formulation (i.e., for Teflon or oil content)? Are there physical or chemical specifications for the final formulation?

6. Is there any physical stability information on the formulation? Has the sedimentation or aggregation of the solid particles been studied as a function of time and temperature? Does the viscosity of the preparation change with time? Are there any other changes that have been observed over time (i.e., color, grittiness, spreadability, etc.)?

Paromomycin Formulation Topical Formulation

Below is further manufacturing description of the optimal paromomycin/gentamicin topical formulation.

Formulation 232 contains:

Paromomycin Sulfate	15.00%[1]
Gentamicin Sulfate	0.50%
Aquaphilic with 10% Carbamide Ointment[2]	67.80%
Water	16.67%

Footnotes:

[1] All percent compositions are weight percent
2 Available from Medco Lab., Inc., Sioux City, Iowa 51103

Aquaphilic with 10% Carbamide Ointment has the following composition:

Sorbitol - 4%
Propylene Glycol - 6%
Water - 39.85%
Urea - 10%
Lactic Acid - 0.5%
Sodium Lauryl Sulfate - 0.75%
Isopropyl Palmitate - 0.5%
Stearyl Alcohol - 19%
White Petrolatum - 19%
Propyl Paraben - 0.15%
Methyl Paraben - 0.25%

Method of Preparation for 150 g of Formulation 232:

1. Weigh 22.5 g of paromomycin sulfate and 0.75 g of gentamicin sulfate.

2. Weigh 101.7 g of Aquaphilic with 10% Carbamide Ointment.

3. Heat 25 ml of distilled water to about 70°C.

4. Heat Aquaphilic with 10% Carbamide Ointment in a separate container to about 70°C.

5. Dissolve paromomycin sulfate and gentamicin sulfate in the heated water with stirring (about 0.5 - 1 min.) and remove solution from heat.

6. Remove Aquaphilic with 10% Carbamide Ointment from the heat and add the aqueous solution of paromomycin sulfate and gentamicin sulfate with stirring. Stir for 10 minutes with an overhead stirrer to incorporate the aqueous solution into the base as it cools.

7. Pour the cooled ointment into an ointment jar. Store at room temperature.

Further Stability Information for Paromomycin Topical Formulations

On August 5, 1992, samples of Formulation 232 and Modified 232 stored at 4°C, 30°C, 37°C and 45°C were sent to WRAIR for animal testing. At that time, the 30°C samples of both formulations showed minor color change and the 4°C samples showed no color change; both had little change in viscosity with the 4°C sample showing a small amount of water separation. The 37°C and 45°C had both shown significant color change for both Formulations 232 and Modified 232; the 232 samples at these elevated temperatures were quite stiff when cooled to room temperature while the Modified 232 samples were more fluid than they were initially.

Samples of both formulations are still stored at 10°C and room temperature (~23-25°C). They have been stored under these conditions for approximately 6 months. Upon inspection, the Formulation 232 showed no color change at 10°C and room temperature and retained its viscosity at both temperatures. There was some water that had separated from the ointment at both temperatures but it could be easily re-mixed. The Modified 232 formulation also showed no color changes after storage at 10 C and room temperature. Viscosity was generally maintained at both temperatures with the room temperature sample developing a harder surface. Presumably this was due to loss of water from the surface of the ointment. This could be minimized by sealing the ointment jar more tightly to reduce evaporation.

Generally both topical formulations appear to hold up well as long as the temperature is maintained at 30°C. There is still a need for a quantitative measure for the stability of paromomycin and gentamicin in these formulations before final assurance of stability can be given. The observed color changes could indicate either small amounts of active component degradation or significant degradation. Without a measure of the antibiotic content in the stored samples it is not possible to choose which stability picture is accurate.

Preparation of Test Batches of Topical Skin Protectant (TSP) Formulations

Small batches (20 gm) of four different TSP formulations were produced and sent to ICD for evaluation. Each formulation was prepared using a laboratory-scale Vertishear homogenizer (Model #225326) in a small scintillation vial. A setting of 10 (speed scale 0 - 100) on the Vertishear homogenizer speed control was used to prepare all formulations. A mixing time of about three minutes was also employed. No difficulties were encountered in preparing these samples. Further consideration of manufacturing aspects of these formulations awaits larger quantities of raw materials with which to make larger batch sizes.

The four-TSP formulations prepared are given below:

Iowa 2377-1

Krytox GPL 102	55% W/W
Teflon MP 1400	45% W/W

Iowa 2305-1

Galden D40	50% W/W
Polymist F5A	50% W/W

Iowa 2423-1

Galden D40	55% W/W
Polymist XPH 284	45% W/W

Iowa Test A

Krytox GPL103	50% W/W
Teflon MP1200	50% W/W

These formulation samples were sent to Dr. Michael McCreery of ICD on 9/15/92 for evaluation.

Production of Antipyrine Capsules

A batch of approximately 4000, 250 mg antipyrine capsules (Batch #WRA-049-11092) were manufactured under FDA's Current Good Manufacturing Practices for use in human clinical studies. These capsules were assayed for content and gave an average content of 249 mg (99.6%) with a relative standard deviation of 2.2%. The disintegration time was < 2 minutes.
This production batch was described in Study Report 333 (2/93).

Progress During Year 2 (2/15/93 - 2/14/94)

Summary

1. Formulation and production of WR238,605 capsules (62.5 mg and 125 mg) and placebo capsules.

2. Formulation development of topical anti-leishmanial ointments continued with the goal of optimizing the most promising formulations.

3. Development work continued on the topical skin protectant formulation (TSP).

4. Development work continued on alkyl aminophenones and alkyl hydroxylaminophenones continued.

These projects are described in more detail below.

1. A batch of approximately 1250 62.5 mg WR238,605 (Lot#141I0393) and 1205 125 mg WR238,605 Lot#142I0393) capsules were produced in March 1993. A batch of 4000 matching placebo capsules (Lot126I0293) were produced in February, 1993. The capsule batch yields were 1205 (96%) for 62.5 mg, 1192 (95%) for 125 mg and 3839 (96%) for placebo capsule batches. The capsule batches were tested for weight variation (placebo only), content uniformity (active batches) and disintegration. The content uniformity's were 99.95% (3.1% RSD) for 62.5 mg and 98.73% (1.1% RSD) for 125 mg batches which met compendial requirements. The placebo capsules had an average weight of 363.58 mg with a relative standard deviation of 1.1%, which meets compendial requirements. The capsules from all batches disintegrated in less than 2 minutes in 900 ml of water at 37°C. The details of these production batches are covered in Study Report #34.

2. Topical anti-leishmanial ointment formulation continued. The most promising formulations contained paromomycin sulfate and gentamicin sulfate in Aquaphilic ointment base containing 10% urea (carbamide). This formulation is designated as WR279,396 and also known as formulation 232. The Aquaphilic ointment with 10% urea is a product of Medco laboratories of Sioux city, Iowa and was used to incorporate paromomycin sulfate/gentamicin sulfate dissolved in aqueous solution. Other formulations included an O/W emulsion base employing nonionic surfactant emulsifiers modified formulation 232 and an aqueous gel formulation prepared with hydroxypropylmethylcellulose. These formulations and studies on these formulations are described in more detail in Study Report #35 (June, 1993). These formulations contained 15% paromomycin sulfate and 0.5% gentamicin sulfate.

3. Development work on the topical skin protectant (TSP) formulation containing fine particles (<10μ) of polytetrafluoroethylene (PTFE) dispersed in a perfluorinated polyether oil (FPE) continued. This work focused on developing methods of preparing test formulations using components from two commercial sources - Ausimont and Dupont.

Formulations containing variations in PTFE and FP content were prepared and sent to ICD for further testing. Methods were also developed for evaluating the TSP formulations to use as quality control procedures to serve as release criteria after manufacture.

4. Preformulation development work on alkyl aminophenones and alkyl hydroxylaminophenones centered on further solubility studies and solution stability studies. such work focused on possible parenteral formulations that would contain optimal concentrations of these amino- and hydroxylamino-phenones for IV or IM injection. Accelerated stability studies were conducted on the best formulations to determine optimal storage conditions and to determine whether any of these amino- or hydroxylamino-phenones would exhibit satisfactory two year stability. The results of this work were reported in Study Report #36 (January, 1994). Important solubility and analytical data from this report are given below.

Solubility of p-Proprioaminophenone in Various Solvents

Solvent	Solubility calculated from UV data at 232 nm (mg/mL)	Solubility calculated from UV data at 316 nm (mg/mL)
Water	0.32	0.34
95% Ethanol	26.96	26.90
Propylene Glycol	15.87	16.51
PEG 200	70.28	71.74
PEG 400	87.00	88.06
Triacetin	22.72	20.05

Solubility of p-Heptanoaminophenone in Various Solvents

Solvent	Solubility calculated from UV data at 233 nm (mg/mL)	Solubility calculated from UV data at 317 nm (mg/mL)
Water	0.002	0.011
95% Ethanol	134.7	132.1
Propylene Glycol	45.4	47.3
PEG 200	126.6	125.3
PEG 400	124.6	124.4
Triacetin	82.0	78.7

Solubility of p-Octanoaminophenone in Various Solvents

Solvent	Solubility calculated from UV data at 233 nm (mg/mL)	Solubility calculated from UV data at 316 nm (mg/mL)
Water	0	0
95% Ethanol	74.3	74.7
Propylene Glycol	23.0	23.4
PEG 200	101.0	102.2
PEG 400	125.8	126.8
Triacetin	46.3	41.8

Analysis of Hydroxyaminophenone Solutions in Propylene Glycol

Objective - Propylene glycol solutions of heptano- (PAHP-OH) and octano-hydroxylamino-phenone (PAOP-OH) were submitted by WRAIR for analysis. Analysis was performed by UV spectroscopy.

Methods - Samples of PAHP-OH and PAOP-OH in propylene glycol, submitted by WRAIR, were used as received. These solutions were turbid and were analyzed unfiltered and after filtration. Millex-GV, Durapore filters (0.22 μ) were used for the for the solutions labeled "filtered" or "unfiltered," "1 mg/mL" and "10 min" or "24 hr." Nylon Acrodisc filters (0.45 μ) were used for the other samples which had been prepared over 6 weeks ago. Aliquots (100 μL) of these solutions were diluted with 95% ethanol to 10 ml to give a 100-fold dilution. This brought the solutions to a nominal concentration of 10 μg/mL.

The diluted samples were analyzed by scanning the solutions from 200-400 nm on Hewlett-Packard 8450A diode-array spectrophotometer. Standards of PAHP-OH (10.07 μg/mL) and PAOP-OH (10.08 μg/mL) were prepared in 95% ethanol and scanned over the same wavelength range. Concentrations of the samples were calculated by ratioing the standard absorbencies at 310 nm to those obtained for the samples.

Results - There were substantial differences in sample concentrations between unfiltered and filtered solutions. There were also substantial shifts in the wavelength maxima and the overall shapes of the spectra that may indicate instability.

Conclusions - These solutions appear unstable as evidenced by their turbidity, shift in wavelength maxima and lower than expected concentrations. The most recently prepared samples have spectra that most closely conform to that of the standards. Also, after filtration, the spectral maximum shifts toward that of the standard. The most freshly prepared samples had UV maxima that were identical to that of the standard. This suggests that the insoluble material in these samples may be predominately degraded material.

Further investigation of the stability of these hydroxaminophenones is warranted to determine how quickly they degrade in solution. It will also be important to determine how the solvent and storage temperature affect stability.

Solubility of Hydroxyaminophenones in PEG 200

The solubility of the heptano-hydroxy-derivative (WR 272677AA) and the octano-hydroxy-derivative (WR 271159AA) in PEG 200 have been further investigated.

The amount dissolved was determined by adding 25 mg of each compound to separate 5 ml aliquots of PEG 200 (Sigma, Cat.# P-3015) in a screw cap culture tube. The tube was sealed and allowed to rotate at room temperature overnight. The solutions were filtered through 0.22 μ solvent-resistant filters (Millex-GV). The filtrate was diluted 500-fold with 95% ethanol. The diluted solution was analyzed by UV spectroscopy and compared to the absorbance of a freshly prepared standard.

It was found that the heptano-hydroxy-derivative gave a concentration of 3.96 mg/mL and the octano-hydroxy-derivative gave a concentration of 4.54 mg/mL. It should be noted that these are not solubilities but rather the actual concentrations obtained after a solution at a nominal concentration of 5 mg/mL was prepared. There is significant insoluble material that is removed by filtration. It is possible that the solubilities of both compounds may be many-fold higher than these concentrations. Since the hydroxy-derivatives are in short supply we have not tried to attain saturation in organic solvents like PEG 200, propylene glycol or ethanol.

There is more evidence that these compounds are unstable in solution at room temperature. Standards of both hydroxy-derivatives in 95% ethanol show progressive shifts in their UV spectral maxima to higher wavelengths. The precipitates found in stored solutions appear to be degradation products. There is less confidence that these solutions can be stored for any length of time and maintain complete stability. It is suggested that any hazy or turbid solution be filtered before use and the resulting clear solution analyzed as soon as possible.

We have kept 25 ml of this solution and will place 1-2 mL aliquots at various temperatures (37°C, RT, 4°C, -20°C and -70°C) and followed the change in spectrum over time.

Progress during Year 3 (2/15/94-2/14/95)

1. Preformulation development alkyl aminophenones and alkyl hydroxylaminophenones continued that was initiated in year 1 and continued in year 2 of this contract.

2. Formulation and production of 250 mg WR238,605 capsules and placebo capsules was performed

3. Formulation and production of 5 mg, 15 mg and 30 mg WR6026 capsules was performed.

4. Development work continued on the topical skin protectant formulation (TSP).

These projects are described in more detail below.

1. Preformulation development work on alkyl aminophenones and alkyl hydroxylaminophenones was completed. The compounds studied were proprio- (PAP), heptano- (PAHP) and octano- (PAOP) aminophenones and their equivalent hydroxylamino derivatives (proprio-, PAPP-OH), octano-, PAOP-OH); heptano-, PAHP-OH). Solubility and stability data were obtained on these compounds in various solvents to support efficacy and toxicity testing in animals. Solutions of these compounds were also prepared and sent to WRAIR and/or the University of Illinois for efficacy or toxicity testing in animals. Procedures were developed to obtain reproducible assay results in viscous solvents like PEG200, PEG400 and propylene glycol. Solutions of these compounds in such solvents were difficult to assay reproducibly because of their viscous nature. Different assay results were obtained with different pipetting techniques which could lead to variable results between different laboratories. Also, the stability of these compounds in solution was marginal. In particular, the alkyl hydroxylaminophenones had the poorest solution stability compared to their alkyl aminophenone equivalents. The purity of the alkyl hydroxylaminophenones did not seem to be as high as that of the alkyl aminophenones. This may have contributed to their lower apparent solubilities and poorer solution stability. Suspension and capsule formulations of these compounds were also developed for oral administration. Further details of this development work are described in Study Report #36 (March, 1994).

2. Production of a batch of 1148 capsules of (theoretical-1200) of 250 mg WR238,605 (Lot #231I0594) was completed and a batch of 1000 matching placebo capsules (theoretical-1000 Lot #251I0594). The content of the 250 mg capsules was an average of 242.8 mg (97%) with a relative standard deviation of 1.6%. The placebo capsules had an average weight of 373 mg with an RSD of 3.5%. Both capsule batches met USP requirements for content uniformity or weight variation. Both capsule batches had an average disintegration time of less than 3 minutes in 900 ml of water at 37°C. These capsules were produced in #1 white opaque capsule shells and packaged in units of 50 capsules in plastic bottles. Further description of the manufacturing procedures, formulation components and quality control test results are in Study Report #37 (July, 1994).

3. Formulation and production of three batches of WR6026 were performed. The strengths were 5 mg (Lot#302I0794), 15 mg (Lot#303I0794) and 30 mg (Lot #304I0794). The batch sizes were 1108 (theoretical-1500) for the 5 mg capsules, 7071 (theoretical-1500) for the 15 mg capsules and 2541 (theoretical-3000) for the 30 mg capsules. The content for the 5 mg capsules was a mean of 4.87 mg (91.4%) with a relative standard deviation (RSD) of 3.3%; the 15 mg capsules had an average content of 14.03 mg (93.5%) with an RSD of 4.0%; the 30 mg capsules had an average content of 30.65 mg (102.1%) with an RSD of 4.3%. All batches met the USP requirement for content uniformity. Further description of the formulation components, manufacturing procedure and quality control testing are contained in Study Report #38.

4. Development work on the topical skin protectant (TSP) formulation containing fine particles (<10μ) of polytetrafluoroethylene (PTFE) dispersed in a perfluorinated polyether oil (FPE) was finalized. This work developed methods of preparing test formulations using components from two commercial sources - Ausimont and Dupont. Procedures were developed to manufacture TSP formulations in a GMP manufacturing facility. Quality control test methods were also developed for evaluating the TSP formulations to serve as release criteria after manufacture. The Haake RS-100 rheometer was purchased for evaluating the visco-elastic properties of TSP formulations to better understand their rheological behavior. The development of rheological test procedures was begun during this budget year.

Progress During Year 4 (2/15/95-2/14/96)

During the budget the following projects were initiated or completed:

1. Production of one batch of 15% paromomycin sulfate/0.5% gentamicin sulfate ointment (WR279,396), one batch of placebo ointment and one batch of white petrolatum.

2. Further development of topical skin protectant (TSP) continued with rheological, content analysis and stability tests being further refined.

These projects are described in more detail below:

1. The active 15% paromomycin sulfate/0.5% gentamicin sulfate ointment (WR279,396, Lot#268I1095) was in a 500 gram batch size. This ointment was prepared in a commercial ointment available from Medco, Inc. as Aquaphilic Ointment with 10% Urea. The placebo ointment (Lot#275±1095) was prepared in the same batch size with sufficient water added to produce an ointment with the same viscosity and spreadability as the active formulation. both ointment batches were packaged in 6 gram quantities in 15 ml wide-mouth amber glass vials sealed with 28mm single piece aluminum seals that have an inner polymeric coating. White petrolatum, USP, was also packaged in 6 gram quantities in the same amber vials to serve as a control (Lot#276I1095). No testing was performed on these ointment formulations.

2. Topical skin protectant development focused on evaluating the rheological properties of TSP test formulations. To this end a controlled stress viscometer (Haake RS-100) was purchased and employed to evaluate test formulations by oscillatory and creep test methods. The oscillatory testing permitted the extraction of viscous and elastic module from rheological data. This allowed the estimation of viscoelastic properties of test formulations, which continuous shear viscometers, could not do. The creep test further evaluated the viscoelastic behavior of TSPs under extremely low shear conditions. These conditions permitted estimation of physical stability characteristics of TSPs like sedimentation. Such rheological data under various stress conditions were also variable in assessing the shear conditions for TSP preparation and how long it would take for a TSP to reach equilibrium viscoelastic behavior.

   Additional effort was expended in developing quality control procedures for determining the level of polytetrafluoroethylene (PTFE) in the fluorinated polyether ether oil (FPE). Reasonably accurate, though not specific, gravimetric methods were developed to first separate and then measure the PTFE and FPE components of TSPs. Accelerated physical stability testing was investigated by centrifugation. It was assumed that the quantity of FPE separation under centrifugation would parallel long term sedimentation behavior of test TSP formulations.

Progress During Year 5 and Extension (2/15/96-2/28/98)

During this budget year the following projects were initiated or completed:

1. Production of one batch of WR238,605 capsules (125 mg, Lot #089I0496) and matching placebos (Lot #088I0496).

2. Preformulation studies on halofantrine HCl and WR178,460 HCl.

3. Production of one batch WR238,605 capsules (250 mg, Lot #040I0297) and matching placebos (Lot#039I0297).

4. Preformulation studies on WR242,511.

5. Production of 15% paromomycin sulfate/0.5% gentamicin sulfate topical ointment (WR279,396, Lot #258I0996) and placebo (Lot #257I0996).

6. Production of 15% Paromomycin sulfate/0.5% gentamicin sulfate topical ointment (WR279,396, Lot #038I0297) and placebo (Lot #037I0297).

These projects are described in more detail below.

1. WR238,605 succinate (Lot AJ, Bottle Number - BM12562) was produced in a 125 mg capsule strength in a batch size of 7,225 (theoretical batch size - 7,985). This lot was #089I0496. A matching placebo (Lot #088I0496) was produced in a batch size of 4603 (theoretical batch size - 5,000). The capsules were tested for weight variation, disintegration time and content uniformity and met USP 23 requirements, where applicable. The active and placebo capsules were shipped to WRAIR on March 3, 1996. The results of this formulation project are described in Study Report 37 (July, 1996).

2. Preformulation studies on halofantrine HCl and its desbutyl derivative (WR178,460 HCl) were conducted. The goals of this work were to compare the solubilities of both compounds in various media and determine their pK_a values. This preceded anticipated further formulation development of WR178,460 HCl for animal and human trial. The results of this work are described in Study Report 38 (June, 1996).

3. WR238,605 succinate (Lot AU, Bottle Number - BN69548) was produced in a 250 mg capsule strength in a batch size of 7334 (theoretical batch size - 7532). This lot was #040I0297. A matching placebo (Lot#039I0297) was produced in a batch size of 14720 (theoretical batch size - 15433). The capsules were tested for weight variation, disintegration time and content uniformity and met USP 23 requirements, where applicable. The active and placebo capsules were shipped to WRAIR on March 3, 1997 during the first quarter of the following year. The results of this formulation project are described in Study Report 40 (May, 1997) which was prepared in the first quarter of the following year.

4. Preformulation studies on WR242,511 tartrate (Lot AF, Bottle Number - BM19356) were initiated during this budget year and completed during the first quarter of the following year. The goals of this work were to determine the basic physicochemical characteristics of this investigational drug. The studies included characterizing the solid-state properties of this drug by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), powder X-ray diffraction, Fourier transform infrared spectroscopy and thermogravimetric analysis (TGA). Drug-excipient compatibility studies were conducted by DSC with possible excipients that could be used in solid formulations. Analytical methods for assay of WR242,511 in formulations and to assess its stability were developed. These assays included UV spectral and HPLC analytical methods. The solution properties of this drug were also investigated - these included acid-base characteristics, solubility in various media and stability under various temperature, pH and light. This work preceded anticipated further formulation development of WR242,511 HCl for human trials. The results of this work are described in study Report 39 (May, 1997) which was prepared in the first quarter in the following year.

5. A topical formulation of 15% paromomycin sulfate/0.5% gentamicin sulfate in Aquaphilic ointment with 10% urea (WR279,396) in a quantity of 65 x 50 g jars. This was lot number #258I0996. A placebo formulation (Lot #257I0996) was also produced in the same quantity. After storage of this formulation, it was found that this product had unsatisfactory properties and was not used in further clinical tests.

6. Another topical formulation of 15% paromomycin sulfate/0.5% gentamicin sulfate in Aquaphilic ointment with 10% urea (WR279,396) was produced to replace that from above. This formulation was prepared by preparing the ointment base from its components rather than using commercial Aquaphilic ointment with 10% urea. The lot yielded 4.76 kg (theoretical batch size - 5 kg) of active ointment which was packaged in 4 oz ointment jars (50 g each) which yielded 95 jars. This was lot number #038I0297. A placebo formulation (Lot#37I0297) was also produced in a batch size of 2.47 kg which was filled into 4 oz ointment jars (50 g each) which yielded 48 jars. These formulations were shipped to Walter Reed Army Institute of Research on February 19, 1997.

Rec'd 1/11/2000

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 4 Jan 00

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCA, 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for the attached Grants. Request the limited distribution statements for Accession Document Numbers listed be changed to "Approved for public release; distribution unlimited." This report should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by email at Judy.Pawlus@amedd.army.mil.

FOR THE COMMANDER:

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

'92-C-2035 AD-B243211

Completed 1-14-00 a